THE CONTRACTILE VACUOLE IN AMOEBA PROTEUS (LEIDY)

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Metcalf (1910) maintains that there are scattered through the cytoplasm of *Amoeba proteus* numerous small round granules called microsomes or cytomicrosomes, and that the "contractile vacuole is surrounded by a layer of granules of the same size and appearance as the microsomes of the general cytoplasm." He says (p. 302): "When the vacuole is of moderate size these granules form a continuous layer, one granule thick," when it is large there are open spaces between the granules, and when it is small the layer is several granules thick. He maintains that during contraction these granules form a clump in which the new vacuole develops and consequently concludes that they "are connected functionally with excretion," and he therefore calls them "excretion granules." He admits, however, that the contractile vacuole can arise and function without the granules. He says (p. 307); "The results of operations upon Amoeba show that a new vacuole in an Amoeba fragment appears first and that only gradually the granules collect about it. The granules are not essential to the functional vacuole."

Mast (1926) confirmed Metcalf's contentions concerning the form, size and distribution of the granules in question, but he holds that the conclusion that they function in excretion is not well founded and that their aggregation on the surface of the contractile vacuole is a purely physical phenomenon. He designated them beta granules to differentiate them from somewhat similar but much smaller granules which he designated alpha granules.

Mast and Doyle (1935*a*, *b*) demonstrated that the beta granules have staining properties like the granules in other cells, known as mitochondria. They centrifuged amoebae and found that all the beta granules moved through the cytoplasm from the axis of rotation and that those on the contractile vacuole moved to the centrifugal surface and formed a considerable mass there, and they obtained some evidence indicating that the beta granules on the surface of the contractile vacuole are imbedded in a layer of substance which is somewhat more viscous than the adjoining substance in the cytoplasm and moves with them during the process of centrifuging. They conclude that the beta granules function in transferring substances from place to place in the cytoplasm and that they probably thus facilitate excretion of substance by the contractile vacuole.

Methods

I made at various times numerous detailed observations on the contractile vacuole in *Amoeba proteus* under the best optical system obtainable (oil immersion apochromatic objective, 60 × and 90 ×, and compensating oculars, 10 ×, 15 × and 20 ×). All the observations were made on living specimens. Some of the specimens had been without food for several days and contained no food vacuoles and but little fat and relatively few granules and crystals. Some of these were greatly flattened by gradually removing water from under the coverglass. Under these conditions the contractile vacuole and the granules and other substances around it could be very clearly seen. The following results were obtained.

Results

The contractile vacuole is usually surrounded by a layer of beta granules (Metcalf's excretory granules). These granules are imbedded in a layer of substance which is optically distinctly different from the adjoining cytoplasm. It has a slightly yellowish tint and appears to consist of coagulated substance. It is more viscous and heavier than the rest of the cytoplasm for it moves to the centrifugal surface of the contractile vacuole when amoebae are centrifuged (Mast and Doyle, 1935*b*, Fig. 1). When the contractile vacuole is fully developed the granules around it usually form a layer one granule thick, with some spaces between the granules here and there. As the vacuole contracts these spaces disappear, the layer of granules becomes continuous and the layer of substance in which they are imbedded thickens. As it contracts further this layer becomes still thicker and the granules pile up on each other until there is, when contraction is complete, a distinct mass of substance of considerable size, in which a remnant of the vacuole can usually still be seen. That is, the vacuole usually does not completely disappear during systole and soon begins to enlarge again. If it disappears completely a new vacuole usually appears and develops in the mass of substance in which the old one disappeared (Fig. 1). This, with the exception of the layer of substance in which the granules are imbedded, is in full accord with Metcalf's views (1910).

At the surface of the contractile vacuole under the layer of substance containing the beta granules, there is a layer or membrane about

0.5μ thick which is optically well differentiated from the adjoining substance on either surface, for under favorable conditions a line indicating an interface can be clearly seen at both of these surfaces. The beta granules are never in close contact with this membrane.

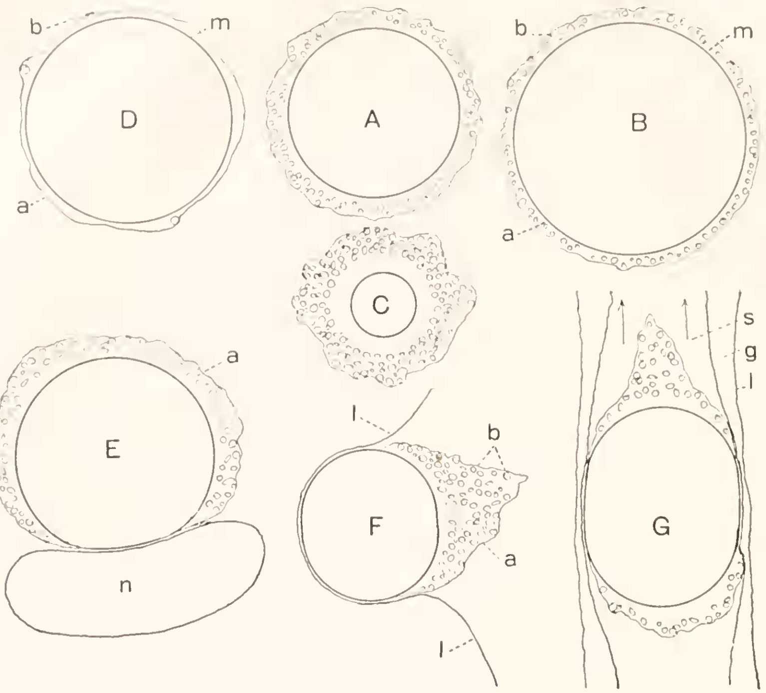

FIG. 1. Camera drawings of the contractile vacuole in *Amoeba proteus* showing the relation between the vacuole and the surrounding cytoplasm, under different conditions and in different phases. *m*, membrane on the surface of the vacuole; *a*, layer of differentiated cytoplasm around the vacuole; *b*, beta granules (mitochondria) imbedded in this layer; *l*, plasmalemma; *g*, plasmagel; *s*, plasmasol; *n*, nucleus.

A, contractile vacuole partially expanded; *B*, same vacuole fully expanded; *C*, same vacuole shortly after contraction; *D*, a vacuole which had only a few beta granules in the differentiated layer at the surface; *E*, *F*, *G*, contractile vacuoles which are in contact with other structures.

Note that the beta granules are not in contact with the membrane at the surface of the vacuoles, that they differ greatly in number in different vacuoles, and that the layer of differentiated cytoplasm in which they are imbedded becomes much thicker as the vacuole contracts. Note also that when the contractile vacuole comes in contact with other structures the differentiated layer around the vacuole is pushed aside.

They are always separated from it by substance in the layer in which they are imbedded. This separation is least when the contractile vacuole is fully developed and increases as it contracts (Fig. 1).

If the contractile vacuole comes in contact with an object, the layer in which the beta granules are imbedded is pushed aside and the membrane around it comes in direct contact with the rest of the cytoplasm (Fig. 1). This shows that this layer is only loosely connected with the membrane.

The number of beta granules varies greatly and probably also the amount of substance in which they are imbedded. I have seen a considerable number of fully developed vacuoles which functioned normally in all respects, with only 10 or 12 granules at the surface (Fig. 1), and one in which there were no granules and no differentiated layer of substance. This indicates that these granules and the layer of substance in which they are imbedded have but little if any functional relation with the contractile vacuole. The layer of substance is probably due to the action of the fluid in the vacuole on the adjoining cytoplasm.

Discussion

There is considerable confusion in reference to the ideas held by different investigators concerning the designation of the structure and the function of the granules and crystals in the cytoplasm of the protozoa.

Bütschli (1881, Abt. 1, S. 103) maintains that there are in fresh-water rhizopods and other protozoa, numerous highly refractive bodies ("Körner") which vary greatly in size "von ausserster Kleinheit bis zu ziemlich ansehnlichen Dimensionen," and frequently appear in the form of rhomboid crystals, and that they are not composed of fat. He holds that these bodies are end-products of metabolism and he calls them "Excretkörnchen." He obviously, therefore, includes under this term various kinds of granules and crystals, that is, all which are highly refractive except those which are composed of fat.

Schewiakoff (1893) found in *Paramecium* small bodies he called "Exkretkörner." He studied these bodies in considerable detail and concluded that they consist of calcium phosphate and are optically active, "doppeltbrechend im polarisirten Lichte." He says (p. 32) "In Folge der Doppeltbrechung, welche bereits von Maupas ermittelt wurde, sind sie von anderen Plasmaeinschlüssen leicht zu unterscheiden." Schewiakoff's Exkretkörner therefore include only a portion of Bütschli's Excretkörnchen, in fact only a very small portion, for only a very small percentage of the granules and crystals in *Amoeba proteus* are optically active.

Metcalf (1910), as previously stated, maintains that there are on the surface of the contractile vacuole and scattered through the cytoplasm of *Amoeba proteus* small round granules. He calls them "microsomes," "Cytomicrosomes," or "excretory granules." Mast and

Doyle (1935) demonstrated that these granules are not optically active. They are therefore not like the Exkretkörner of Schewiakoff.

Taylor (1923, p. 266) says that certain granules which appear throughout the cytoplasm of *Euplotes* are apparently comparable with Metcalf's "cytomicrosomes" in *Amoeba* and *Opaline* (1910), Bütschli's "Exkretkörner" (1881), Maupas' "corpuscle refringents" (1883), and Schewiakoff's "Exkretkörner" in *Paramecium*.

Since Metcalf's cytomicrosomes differ radically from Schewiakoff's Exkretkörner, it is difficult to see how the granules in *Euplotes* can be comparable with both. Taylor does not say whether or not they are optically active but he calls them "crystaloidal, endoplasmic granules" and he says "I have never observed that they tend to aggregate about the contractile vacuole." They are therefore probably more nearly like Schewiakoff's Exkretkörner than Metcalf's "cytomicrosomes" or "excretory granules."

Taylor (p. 278) maintains that Khainsky (1911) observed granules pass through the walls of the food vacuoles into the cytoplasm and he says: "These crystalloids he [Khainsky] identifies with the Exkretkörner described at length by Schewiakoff. Khainsky thinks that eventually the granules are dissolved in the endoplasm and the solution is discharged to the outside by the contractile vacuole." I find that Khainsky presents evidence which indicates that granules pass from the food vacuoles directly into the surrounding cytoplasm, but I find nothing which indicates that he identified these granules with Schewiakoff's Exkretkörner or that he held that they are dissolved in the endoplasm and discharged through the contractile vacuole.

The fact that Bütschli designated the granules and crystals he observed "Excretkörnchen" clearly indicates that he held that they are involved in excretion. He says nothing, however, concerning this.

Schewiakoff maintains that the bodies he designated "Exkretkörner" dissolve in the cytoplasm and are excreted in fluid form by the contractile vacuole. He says (p. 55): "Es liegt demnach die Vermuthung nahe, dass die Exkretkörner im Protoplasma aufgelöst und im füssigen Zustande durch die kontraktile Vacuole nach ausen entleert werden."

Metcalf (1910) thinks that his "excretory granules" are "functionally connected with excretion." He gives nothing concerning the method involved, but his description of the relation between these granules and the action of the contractile vacuole shows clearly that he holds that they are not dissolved and discharged by the contractile vacuole. His conception concerning the method of function of these granules consequently differs radically from Schewiakoff's concerning the method of function of his Exkretkörner.

Taylor (1923, p. 277) says: "Vacuoles (contractile) in Euplotes make their first appearance either from the coalescence of other extremely minute vacuoles that easily escape detection, or from the transformation of small vacuoles which contain one or more granules ('Exkretkörner'), or they arise de novo." I can see no essential difference between the first possibility and the third.

Concerning the second possibilities, Taylor thinks the granules in question may pass from the food vacuoles into the cytoplasm in accord with Khainsky's observations referred to above, that vacuoles may thus form around these granules, that the granules may then dissolve, and that the fluid vacuoles thus formed may develop into contractile vacuoles; and he holds that if this obtains the origin of the contractile vacuole is traceable to the food vacuole. The fact, however, that the contractile vacuoles continue to operate normally for days in the total absence of food, that long after all vacuoles containing food and the granules which have passed from them into the cytoplasm have disappeared, practically proves that they do not originate in this way.

Concerning the third possibility Taylor says: "Now it is conceivable that here and there in this organism 'fluid centers' normally arise which contain in solution substances (e.g. catabolic products) of a kind and concentration sufficient to induce the gelation of the surrounding plasm, thereby forming de novo normal vacuoles."

This possibility is in full accord with Metcalf's views concerning the origin of the contractile vacuole, except that he holds that the beta granules ("excretory granules") are involved. The fact, however, that under some conditions the contractile vacuole forms in regions where there are but few if any of these granules and that it develops normally at times when there are practically none on the surface of it, demonstrates that if they are at all functionally connected with the origin or the development of this vacuole, it is only in a very minor way.

It may be said then that while it is highly probable that the contractile vacuole originates in minute localized aggregations of fluid in the cytoplasm, nothing is known concerning the factors involved.

There is no evidence concerning the function of the relatively viscous layer in which the beta granules are imbedded on the surface of the contractile vacuole.

Summary

1. The contractile vacuole in *Amoeba proteus* contains at the surface a well differentiated membrane about 0.5μ thick. Adjoining this

membrane on the outside there is usually a layer of substance in which numerous beta granules (Metcalf's "excretory granules") are imbedded. This layer is more viscous and heavier than the adjoining cytoplasm. It is usually about 3μ thick when the vacuole is maximum in size and it becomes thicker during contraction.

2. The beta granules around the contractile vacuole vary greatly in number and the layer of substance in which they are imbedded varies greatly in thickness, without any apparent variation in the function of the vacuole. These facts indicate that neither the granules nor the layer of substance is involved in the function of the contractile vacuole, at least not directly.

3. The differentiation of a layer of substance on the surface of the contractile vacuole is probably due to the action of the fluid in the vacuole on the adjoining cytoplasm.

4. There is much confusion concerning the ideas held by different investigators in regard to the nomenclature, the structure and the function of the various granules and crystals in the cytoplasm of the protozoa.

5. Bütschli's "Excretkörnchen" consist of various different kinds of granules and crystals found in protozoa. Schewiakoff's "Exkretkörner" are optically active bodies, nearly all of which, if not all, are crystals. Metcalf's "excretory granules" are not optically active. They are approximately 1μ in diameter and have staining properties like the mitochondria in other cells. The "Excretkörnchen" of Bütschli, the "Exkretkörner" of Schewiakoff and the "excretory granules" of Metcalf therefore differ radically in structure.

6. Bütschli says the "Excretkörnchen" are end products of metabolism but he says nothing concerning their elimination. Schewiakoff contends that the "Exkretkörner" are formed in the food vacuoles, pass out into the cytoplasm, dissolve there and are eliminated by the contractile vacuole. Metcalf holds that the "excretory granules" (Mast's beta granules) are permanent structures which function in excretion of substance by the contractile vacuole. Mast and Doyle maintain that they function in transporting substance through the cytoplasm.

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